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Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity

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Abstract

Barley is one of the most salt tolerant crop species, and differences between barley genotypes for salinity tolerance have been previously documented. Greenhouse experiments were conducted with barley seedlings (up to fourth leaf) from 14 genetic lines grown in control and saline (EC = 20 dS m^{-1}) conditions. Some of these barley genotypes are parental lines to diverse mapping populations. Others have been bred and released for their tolerance to salinity. Gas exchange, chlorophyll fluorescence parameters, above ground dry matter and carbon isotope discrimination were measured to determine salinity tolerance. Two-week exposure to saline conditions decreased above-ground dry mass (AGDM), net photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i), efficiency of light harvesting of photosystem II (F'_v/F'_m), photochemical quenching (q_P), and carbon isotope discrimination (Δ) relative to control plants. Measurement of g_s provided the best information to assess genetic differences in barley for absolute performance when subjected to salinity stress. Lines with the highest g_s values under control conditions also showed some of the highest absolute values for A and F'_v/F'_m under saline conditions. All lines were enriched in ^{13}C (lower Δ) with salinity, but Δ was of limited value to assess differences between lines. Salinity susceptibility indexes (SSI) were used to estimate the relative tolerance of lines to salinity. They varied considerably between parameters and provide only relative information that can be difficult to reconcile with above absolute values of performance under saline conditions.

Keywords: Barley; Carbon isotope discrimination; Chlorophyll fluorescence; Gas exchange; Salinity tolerance

1. Introduction

Excessive salinity from soils or irrigation water poses major challenges to crop production around the world (Tanji, 1990; Flowers, 2004). In many regions of the world and many areas of the Intermountain Region of the USA, salinity stress may occur when crops are exposed to high levels of Na and Ca salts. The effects of Ca salts are often overlooked, though their effects may be greater than those of sodium salts (Aceves et al., 1975). In this comparative study of salinity tolerance among barley lines, the effects of these two salt species were not separated but, rather, assessed as a combined effect. Similarly to other abiotic stresses (i.e., drought, heat, and chilling), salinity is known to negatively affect CO_2 assimilation (Levitt, 1980; Brugnoli and Lauteri, 1991). Stomatal (closure of stomata) and non-stomatal (including damage to photosynthetic apparatus) factors may be involved in reduction of CO_2 assimilation (Bethke and Drew, 1992; Kao et al., 2003). Stomatal limitations typically are evaluated using gas exchange. Measurement of chlorophyll fluorescence has been used as a mean to evaluate the integrity of photosystem II upon exposure to stress (Shabala, 2002).

The in vivo effects of salinity on chlorophyll fluorescence have been described for several crop species (Smillie and

Abbreviations: A, net CO_2 assimilation rate; AGDM, above-ground dry matter; C_i, leaf internal CO_2 concentration; E, transpiration rate; F'_m, maximal fluorescence during a saturating flash light; F'_o, minimal fluorescence for a momentarily darkened leaf; F_s, "steady-state" fluorescence; F'_v/F'_m, efficiency of energy harvesting by open reaction centers of photosystem II; g_s, stomatal conductance; q_P, photochemical quenching; iWUE, instantaneous water-use efficiency; Δ, carbon isotope discrimination

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Nott, 1982; Sayed, 2003). Interspecific differences for chlorophyll fluorescence parameters have been documented for soybean (Kao et al., 2003) and *Paspalum* grass (Lee et al., 2004). Fluorescence parameters have been used to screen for salinity tolerance in barley, wheat and corn (Monneveux et al., 1990; Belkhodja et al., 1994; Shabala et al., 1998).

Discrimination between the stable atmospheric carbon isotopes ^{13}C and ^{12}C provides an integrated measure of stomatal control of internal CO_2 concentration. In theory, higher internal CO_2 concentration (C_i) implies higher carbon isotope discrimination (Δ) for the heavier ^{13}C isotope. C_i is dependent on two main parameters: stomatal conductance (g_s) and CO_2 assimilation capacity. Limitation of the former or the latter will lower or increase C_i, respectively. Thus, Δ is considered a predictor of water use efficiency (Farquhar and Richards, 1984). In a number of C_3 species subjected to salinity stress, a decline of Δ values was reported (Brugnoli and Lauteri, 1991; Ouerghi et al., 2000; Rasmuson and Anderson, 2002).

The salt tolerance of barley is among the highest of all crops (Maas and Hoffman, 1977; Levitt, 1980), and differences in salinity tolerance have been reported among barley lines (Epstein and Norlyn, 1977; Rathore et al., 1977; Day et al., 1985; Forster et al., 2000) and barley species (Mano and Takeda, 1998).

Eleven of the 14 tested lines are parental lines to mapping populations currently made available by the North American Barley Genome Program (NABGP) (namely, Harrington, Morex, TR306 and Steptoe) and the National Barley Molecular Marker Program (NBMMP) of Australia (namely, Alexis, Chebec, Galleon, ND11231, Patty, VB9104 and Sloop). Similar populations have been used to initiate genetic mapping of traits linked to salinity tolerance (Mano and Takeda, 1997; Flowers et al., 2000; Forster et al., 2000). Therefore, we decided to test if we could find physiological differences between these eleven parental lines that may be further mapped amongst their progenies. Two other genotypes (AZ-8501, Giza 125) were selected for grain yield under saline conditions (Day et al., 1985; Noaman et al., 1995). Gallatin is a Montana-released cultivar that shows a low level of leaf tip burn injury under saline conditions in Utah field trials (Roche, unpublished results).

The specific objectives of this study were: (i) to ascertain the extent of differences in gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination among 14 barley lines in response to salinity stress; (ii) to elucidate some of the possible reasons for differential physiological responses of these lines to a saline environment; (iii) to determine if any of these parameters may be useful as a selection criterion in breeding barley for tolerance to salinity.

2. Materials and methods

2.1. Plant material and salt treatment

Fourteen genotypes of barley (Table 1; *Hordeum vulgare* L.: Alexis, AZ-8501, Chebec, Gallatin, Galleon, Giza 125, Harrington, Morex, ND11231, Patty, Sloop, Steptoe, TR306 and VB9104) were tested in five identical experiments conducted in a period of three consecutive winter months. In each experiment, each barley line was grown in 14 tube containers (each with a volume of 160 cm^3). Three seeds were planted in sand in each tube. After germination, only one seedling was kept per tube. Seven containers from every barley line were arranged in each of two trays, using a constrained randomization for tube location to control border effects. Tube locations were randomized differently for each experiment. One tray received the control treatment, and the other received the salinity stress treatment. After the second leaf emerged, each tray was immersed every other day for 4 min, either in a nutrient

Table 1
Barley lines used in this study with their respective countries of origin and salinity tolerance

Breeding line	Country of origin	Salinity tolerance	References
Alexis	Germany		
AZ-8501	Arizona, USA	Tolerant	Day et al. (1985)
Chebec	Australia	Unknown	
Gallatin	Montana, USA	Unknown	
Galleon	Australia	Unknown	
Giza 125	Egypt	Tolerant	Noaman et al. (1995)
Harrington	Saskatchewan, Canada	More sensitive than TR306	Mano and Takeda (1997)
Morex	Minnesota, USA	More sensitive than Steptoe	Mano and Takeda (1997)
ND11231	North Dakota, USA	Unknown	
Patty	France	Tolerant[a]	Royo and Aragues (1999)
Sloop	South Australia	Unknown	
Steptoe	Washington, USA	More tolerant than Morex	Mano and Takeda (1997)
		Less tolerant than Patty[a]	Royo and Aragues (1999)
TR306	Canada	More tolerant than Harrington	Mano and Takeda (1997)
VB9104	Australia	Unknown	

Blanks indicate that documentation was not available.
[a] Based on grain yield at EC = 12 dS m^{-1} (see Royo and Aragues, 1999).

solution (Control tray; EC = 3 dS m^{-1}) or in the same nutrient solution containing 1.93 g/L (i.e., 33 mM) of NaCl and 9.83 g/L (i.e., 67 mM) of $CaCl_2 \cdot 2H_20$ (Salinity-stress tray; EC = 20 dS m^{-1}). Plants did not receive any other types of irrigation or nutritive solutions. Greenhouse conditions were maintained at 28/22 °C (day/night) with a relative humidity of 40–60%. Light intensity for plant growth was 800 μmol m^{-2} with a photoperiod of 16/8 light/dark.

2.2. Gas exchange and chlorophyll fluorescence measurements

Net photosynthesis, stomatal conductance, transpiration, efficiency of light harvesting of photosystem II and quenching coefficient were measured at the fourth leaf stage after a total of six solution immersions (14–16 days after beginning of seedling emergence). An infrared, open gas exchange system (LI-6400, LICOR Inc., Lincoln, NE, USA) coupled with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer, LICOR, Inc.) was used to take measurements. All measurements for an experiment were completed in the same day between 10:00 and 16:00 h and were made on five plants per barley line per treatment, omitting two plants at the tray edges to avoid any border effect. Measurement of a control plant was immediately followed by that of the same genotype in the same corresponding location within the salinity-treated tray. The gas exchange system allowed for independent control of CO_2 concentration (by an integrated CO_2 mixer), relative humidity and flow rate set, at 370 μmol mol^{-1}, 15%, and 400 μmol s^{-1}, respectively. The leaf chamber fluorometer was used as a light source. Light intensity was set to 800 μmol m^{-2} s^{-1}, and blue light was set at 10% of the total. Data were manually logged when gas exchange and chlorophyll fluorescence parameters became stable. Values for net CO_2 assimilation rate (A) and intercellular CO_2 concentration (C_i) were calculated using the equations of von Caemmerer and Farquhar (1981). Instantaneous water-use efficiency (iWUE) was calculated as the ratio between net photosynthesis (A) and transpiration (E) (Condon et al., 2002). Fluorescence parameters were measured on light adapted leaves using the equations of Genty et al. (1989). The efficiency of energy harvesting by open reaction centers of photosystem II for light-adapted leaf was calculated as:

$$F'_v/F'_m = (F'_m - F'_o)/F'_m$$

where F'_o is the minimal fluorescence of a momentarily darkened leaf, and F'_m is the maximal fluorescence during a saturating flash light of >7 mmol m^{-2} s^{-1}.

Photochemical quenching (q_P) was calculated as indicated by the manufacturer's manual for the LI-6400-40 leaf chamber fluorometer.

$$q_P = (F'_m - F_s)/(F'_m - F'_o)$$

where F_s is the "steady-state" fluorescence.

Plants were harvested the day after these measurements were completed to assess above ground dry matter.

2.3. Carbon isotope discrimination

Carbon isotope discrimination was determined in only two experiments (Experiments 4 and 5). The five plants of each barley line from control- and salinity-stressed trays were combined, oven-dried, and ground. Analyses of $^{13}C/^{12}C$ were carried out by isotope ratio mass spectrometry at the Isotope Laboratory, Augustana College, Sioux Falls, SD, USA. Carbon isotope composition was expressed as $\delta^{13}C$ (‰) (Farquhar et al., 1989) computed as [(R sample/R standard) − 1] × 1000, where R was the $^{13}C/^{12}C$ ratio. Precision of the $\delta^{13}C$ measurements was ±0.1‰. Δ was calculated according to Farquhar et al. (1989) as $\Delta = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_a and δ_p refer to air and plant sample, respectively. δ_a (−8‰) is the current deviation of atmospheric CO_2.

2.4. Statistical analysis

After Nogues et al. (1994), a salinity susceptibility index (SSI) for each of several parameters was calculated for each barley line in each experiment as:

$$\text{SSI}\,(\%) = (X_s/X_c) \times 100\%$$

where X_s is the mean value (computed over five plants) of the parameter measured under saline conditions and X_c is the mean value of the parameter measured under control conditions.

The effects of salinity and differences among barley lines in AGDM, A, g_s, C_i, E, F'_v/F'_m, q_P and Δ were assessed using an analysis of variance of a two-way factorial in a complete block design. Each parameter was analyzed individually. Saline conditions and barley lines were fixed-effects factors. Differences among barley lines in SSI for AGDM, A, g_s, C_i, E, F'_v/F'_m, q_P and Δ were assessed using an analysis of variance of a one-way factorial in a complete block design. Each parameter was analyzed individually. Barley line was a fixed-effects factor. For both analyses of variance, experiments were random blocks. Means were computed over the five plants for each combination of barley line and experiment and were used as data values in the analyses. Pair-wise comparisons of barley line means were assessed using a study-wide Type I error rate of 0.05 or 0.10 using the Tukey–Kramer method. All computations were made using Proc Mixed in SAS 9.1 for Windows (SAS Institute, Cary, NC, USA).

Sigmaplot 2001 software (SPSS Inc., Chicago, IL, USA) was used for all the correlations. For the study of relationships between SSI values for g_s and C_i, Pearson correlations were calculated. A best-fit curve of modified simple exponential growth was applied for the correlation between SSI values for F'_v/F'_m and g_s.

Table 2
Observed significance levels (p-values) for effects of salinity treatment, of barley line, and their interaction from analyses of variance for above-ground dry mass (AGDM), net photosynthesis (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration (E), instantaneous water-use efficiency (iWUE), two chlorophyll fluorescence parameters (F'_v/F'_m, q_P) and carbon isotope discrimination (Δ)

Parameter	Treatment	Barley line	Interaction
AGDM	<0.0001	<0.0001	0.0001
A	<0.0001	<0.0001	0.0001
g_s	<0.0001	<0.0001	0.0001
C_i	<0.0001	0.0157	0.3332
E	<0.0001	<0.0001	0.0064
iWUE	<0.0001	0.2492	0.4335
F'_v/F'_m	<0.0001	<0.0001	0.1568
q_P	0.1525	<0.0001	0.5214
Δ	<0.0001	0.0010	0.0362

3. Results

Salinity effects and differences among barley lines were evident for most parameters (Table 2). Effects due to salinity treatment were detected for all parameters except photochemical quenching (q_P). Differences among barley lines were found for all parameters except instantaneous water-use efficiency (iWUE). We found evidence of interaction between barley lines and salinity treatment for above-ground dry matter (AGDM), net photosynthesis (A), stomatal conductance (g_s), transpiration (E) and carbon isotope discrimination (Δ) (Table 2).

3.1. Above-ground dry matter

Under saline conditions, AGDM values were lower (Fig. 1) and relatively similar across barley lines. AGDM values differed significantly only between Patty and Steptoe (Table 3). Relative effects of salinity stress, as measured by a salinity susceptibility index (SSI) (see Section 2) for AGDM, differed among barley lines (Table 4). AGDM for Sloop was less reduced than for either Steptoe or Harrington, and AZ8501 was less reduced than Harrington (Table 4).

3.2. Gas exchange parameters

Salinity reduced net photosynthesis (A), regardless of barley line (Fig. 2a). Under saline conditions, Patty and Sloop exhibited the highest rates for A while Giza 125, AZ-8501, Morex and Steptoe had the lowest ones (Table 3). Indeed, Giza 125 and AZ-8501 also had the lowest values for net photosynthesis (A) under control conditions (Fig. 2a). Giza 125 was the least affected by salinity as indicated by SSI for A (Table 4).

Salinity negatively affected stomatal conductance (g_s) of all lines (Fig. 2b). Under control and saline conditions, Patty and Giza 125 had the highest and lowest values for (g_s), respectively (Fig. 2b, Table 3). Giza 125, AZ-8501 and Sloop were relatively less affected by salinity as indicated by their respective SSI for g_s (Table 4). A differential response to salinity by different cultivars for g_s was reflected by the high number of significant groups between lines (Table 3) and the interaction found for this parameter in the general analysis of variance (Table 2).

Genetics and salinity affected intercellular CO_2 concentration (C_i) though no interaction was found between these effects (Table 2). Using pair-wise comparisons of barley line means (at $p = 0.05$), it is noteworthy that we found four significant groupings of barley lines under control (data not shown) but none under saline conditions (Table 3). All together, barley lines did not differ in their respective SSI values for C_i (Table 4). Nevertheless, the correlation between SSI for g_s and SSI for C_i was positive, indicating that lines most affected by salinity for g_s tended to be most affected for C_i ($r^2 = 0.594$, $p = 0.001$) (Fig. 3).

Across all lines, we found increases of instantaneous water-use efficiency (iWUE) values in saline conditions (Fig. 2d). No differences were detected between barley lines (Tables 2 and 3). Though lines differ significantly in their respective net photosynthesis (A) and transpiration (E) rates, variations for these two parameters were of the same order, and nullified each other in the calculation of iWUE (Table 3). Regarding SSI values for iWUE, only two lines (i.e., ND11231 and Patty) exhibited a significantly higher

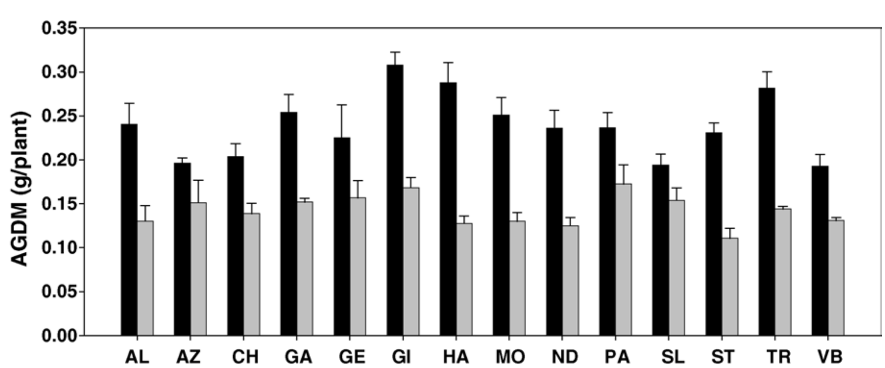

Fig. 1. Above-ground dry mass (AGDM) (g plant^{-1}) for 14 barley lines in control (black bars) (EC: 3.0 dS m^{-1}) and saline (gray bars) (EC: 20 dS m^{-1}) conditions. Means were calculated from five experiments with five individual plants per experiment. Vertical bars represent two standard errors based on variability among 25 plants. Barley lines are abbreviated as follows: AL (Alexis), AZ (AZ-8501), CH (Chebec), GA (Gallatin), GE (Galleon), GI (Giza 125), HA (Harrington), MO (Morex), ND (ND11231), PA (Patty), SL (Sloop), ST (Steptoe), TR (TR306) and VB (VB9104).

Table 3
Observed significance levels (p-values) for overall tests of barley line differences, and pair-wise comparisons of 14 barley lines in saline conditions for above-ground dry matter (AGDM), net photosynthesis (A), stomatal conductance (g_s), transpiration (E), internal CO_2 concentration (C_i), instantaneous water-use efficiency (iWUE), two parameters of chlorophyll fluorescence (F'_v/F'_m, q_P) and carbon isotope discrimination (Δ)

	AGDM (g plant^{-1})	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	C_i (umol mol^{-1})	iWUE (μmol mmol^{-1})	F'_v/F'_m	q_P	Δ
p	0.0183	<0.0001	<0.0001	<0.0001	0.1979	0.3442	0.0023	<0.0001	0.3064
Alexis	0.13 ab	7.0 abcd	0.043 abcdef	1.4 abcd	138.5 a	4.3 a	0.51 a b	0.59 a b c d	16.9 a
AZ-8501	0.15 ab	4.6 ef	0.033 cdefg	1.1 bcde	147.1 a	4.3 a	0.48 b	0.52 cd	16.6 a
Chebec	0.14 ab	7.1 abc	0.051 abc	1.7 ab	144.5 a	4.2 a	0.51 ab	0.61 ab	16.7 a
Gallatin	0.15 ab	5.7 cdef	0.031 defg	1.0 cde	122.4 a	4.6 a	0.51 ab	0.59 abcd	16.5 a
Galleon	0.16 ab	7.0 abcd	0.050 abcd	1.6 abc	149.1 a	4.2 a	0.50 ab	0.61 ab	16.8 a
Giza125	0.17 ab	4.0 f	0.022 g	0.7 e	134.6 a	4.3 a	0.48 b	0.51 d	16.6 a
Harrington	0.13 ab	5.8 bcde	0.034 bcdefg	1.2 bcde	131.4 a	4.4 a	0.50 ab	0.59 abcd	17.1 a
Morex	0.13 ab	4.6 ef	0.027 fg	0.9 de	132.7 a	4.4 a	0.48 ab	0.53 bcd	16.6 a
ND11231	0.12 ab	7.4 abc	0.048 abcde	1.5 abcd	138.8 a	4.5 a	0.52 ab	0.57 abcd	16.5 a
Patty	0.17 a	8.1 a	0.057 a	1.8 a	132.1 a	4.6 a	0.52 a	0.62 a	17.1 a
Sloop	0.15 ab	7.5 ab	0.053 a b	1.7 ab	147.7 a	4.2 a	0.50 ab	0.61 a	17.0 a
Steptoe	0.11 b	5.2 ef	0.031 efg	0.97 cde	141.6 a	4.3 a	0.49 ab	0.53 bcd	16.4 a
TR306	0.14 ab	5.3 def	0.030 efg	1.02 cde	125.9 a	4.5 a	0.48 b	0.57 abcd	16.8 a
VB9104	0.13 ab	5.9 bcde	0.038 a bcdefg	1.21 abcde	145.9 a	4.2 a	0.49 ab	0.60 abc	16.5 a

Values are the means of five experiments for AGDM, A, g_s, F'_v/F'_m and q_P, and of two experiments for Δ. Within a column, mean values with the same letter were not significantly different (Tukey–Kramer mean comparisons at $\alpha = 0.05$).

relative increase than that of Steptoe under saline conditions (Table 4).

3.3. Chlorophyll fluorescence parameters

Efficiency of light harvesting of PSII, as measured by F'_v/F'_m, was generally affected by barley lines and salinity (Fig. 4a). However, AZ-8501 and Giza 125 maintained the same level of F'_v/F'_m in saline and control conditions (Fig. 4a). Under saline conditions, Patty exhibited the highest F'_v/F'_m absolute value (Table 3) differing significantly with those of AZ-8501, Giza 125 and TR306. AZ-8501 had higher SSI value for F'_v/F'_m than Morex, TR306 and VB9104 (Table 4). We found a significant relationship between the sensitivity to salinity (i.e., SSI) of F'_v/F'_m and g_s parameters for all barley lines (Fig. 6), indicating that these parameters may not be independent or are co-regulated. Although we found differences among cultivars for photochemical quenching (q_P) under both conditions, salinity did not significantly affect this parameter (Fig. 3, Table 2), and cultivars did not differ in SSI for q_P (Table 4). Patty and Sloop exhibited the highest absolute values for q_P under saline conditions as AZ-8501 and Giza 125 had the lowest (Table 3).

Table 4
Observed significance levels (p-values) for overall tests of barley line differences, and pair-wise comparisons of 14 barley lines for salinity susceptibility index (SSI) relative to AGDM, net photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i), instantaneous water-use efficiency (iWUE), two parameters for chlorophyll fluorescence (F'_v/F'_m, q_P) and carbon isotope discrimination (Δ)

Genotypes	Salinity susceptibility index (SSI)							
	AGDM	A	g_s	C_i	iWUE	F'_v/F'_m	q_P	Δ
p	0.0032	0.1092	0.2455	0.1319	0.0472	0.0269	0.0485	0.0465
Alexis	58 abc	54 ab	34 a	75 a	121 a b	93 ab	103 a	84 ab
AZ-8501	77 ab	57 ab	42 a	83 a	120 a b	102 a	98 a	82 ab
Chebec	69 abc	53 ab	37 a	74 a	123 a b	97 ab	99 a	84 ab
Gallatin	61 abc	51 ab	33 a	73 a	117 a b	95 ab	102 a	81 ab
Galleon	71 abc	50 ab	36 a	80 a	117 a b	92 ab	92 a	85 ab
Giza 125	55 abc	59 a	41 a	82 a	115 a b	100 ab	100 a	84 ab
Harrington	45 c	48 ab	30 a	73 a	120 a b	93 ab	103 a	82 ab
Morex	53 abc	40 b	25 a	73 a	124 a b	92 b	94 a	81 ab
ND11231	55 abc	53 ab	34 a	70 a	130 a	94 ab	95 a	79 b
Patty	72 abc	55 ab	37 a	69 a	132 a	94 ab	101 a	83 ab
Sloop	79 a	57 ab	41 a	82 a	112 a b	94 ab	101 a	86 a
Steptoe	48 bc	49 ab	34 a	84 a	107 b	94 ab	94 a	83 ab
TR306	52 abc	43 ab	28 a	73 a	116 ab	91 b	96 a	84 ab
VB9104	69 abc	52 ab	36 a	82 a	114 ab	92 b	102 a	86 ab

Values of SSI are the means of five experiments for AGDM, A, g_s, F'_v/F'_m and q_P, and of two experiments for Δ. Within a column, lines with the same letter were not significantly different (Tukey–Kramer mean comparisons at $\alpha = 0.10$).

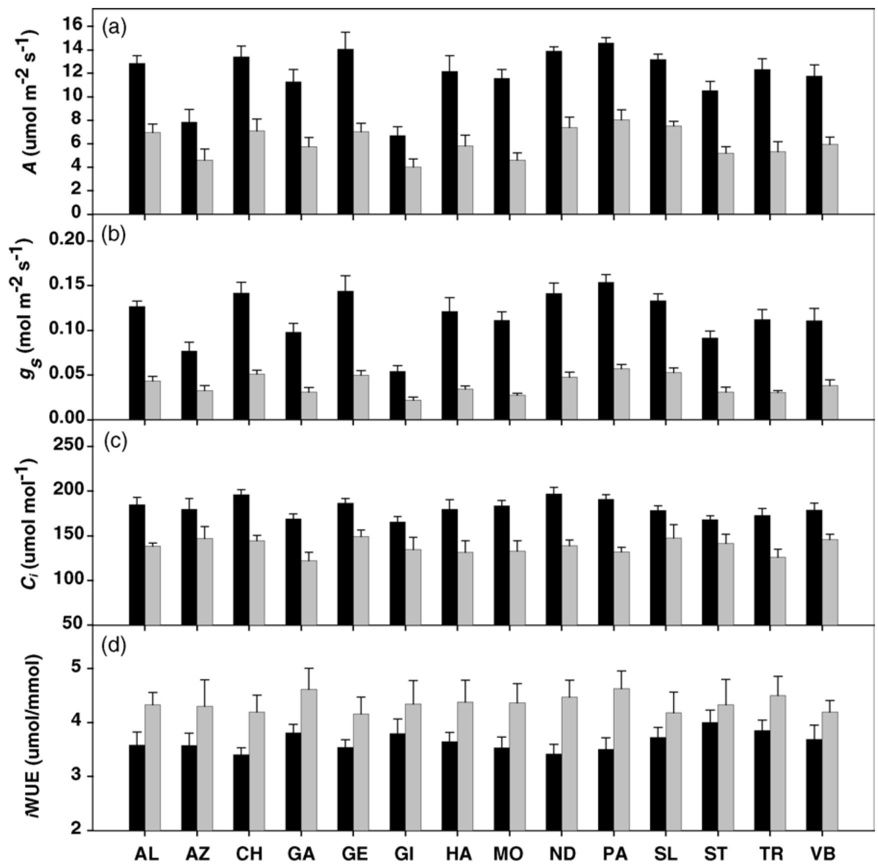

Fig. 2. Mean gas exchange parameters of fourth leaf for 14 barley lines in control (black bars) (EC: 3 dS m^{-1}) and saline (gray bars) (EC: 20 dS m^{-1}) conditions. Net photosynthesis (A) on panel a (in μmol CO_2 m^{-2} s^{-1}); stomatal conductance (g_s) on panel b (in mol H_2O m^{-2} s^{-1}); internal CO_2 concentration (C_i) on panel c (μmol CO_2 mol^{-1} air); instantaneous water-use efficiency (iWUE) on panel d (μmol CO_2 mol^{-1} H_2O). Vertical bars represent two standard errors based on variability among 25 plants. Barley line abbreviations are the same as in Fig. 1.

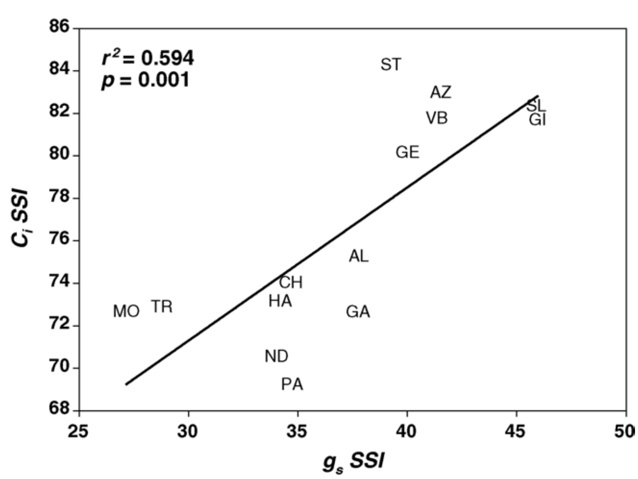

Fig. 3. Relationship between stomatal conductance (g_s) and internal CO_2 concentration (C_i) of fourth leaf for 14 barley lines. Data are the average of five experiments. Values of salinity susceptibility index (SSI) for stomatal conductance and internal CO_2 (both in %) are used for axis. Barley line abbreviations are the same as in Fig. 1.

3.4. Carbon isotope discrimination

Leaves of all 14 barley genotypes grown under salinity were enriched with ^{13}C compared to control plants (i.e., lower Δ values) (Fig. 5). Using pair-wise comparisons of means, we found differences between barley lines in control conditions, with three significantly different groupings (data not shown), and none in saline conditions (Table 3). Δ is an integrated value for carbon metabolism over the whole development of these young barley plants. On the contrary, measurements of gas exchange and chlorophyll fluorescence are instantaneous in nature. We studied the relationship between Δ and each of the gas exchange and fluorescence parameters to assess whether intensive instantaneous measurements may be replaced by easier measurements of carbon isotope discrimination. We found no significant correlation between Δ and any of the gas exchange and fluorescence parameters for plants grown under control and saline conditions (data not shown).

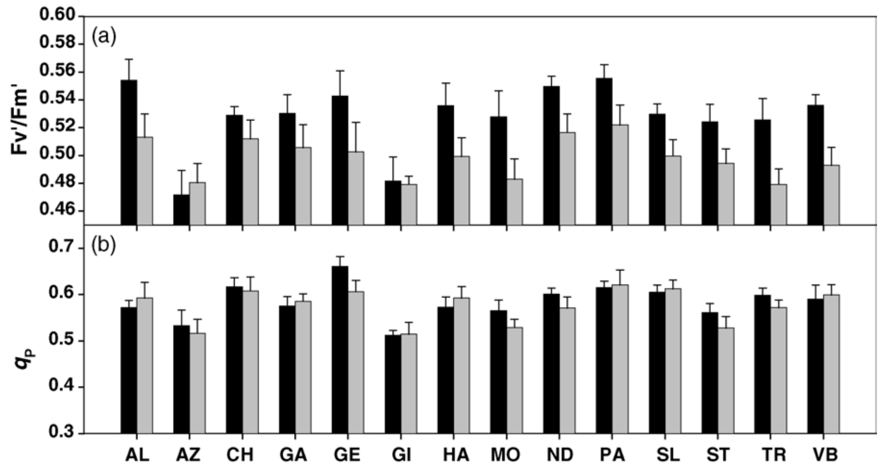

Fig. 4. Means for chlorophyll fluorescence parameters of fourth leaf for 14 barley lines in control (black bars) (EC: 3 dS m^{-1}) and saline (gray bars) (EC: 20 dS m^{-1}) conditions. Fraction of absorbed photons used in photochemistry (F'_v/F'_m) on panel a; photochemical quenching (q_P) on panel b. Vertical bars represent two standard errors. Barley line abbreviations are the same as in Fig. 1.

4. Discussion

In barley, as in other species, tolerance to salinity may differ with developmental stages (e.g., germination versus seedling growth versus vegetative growth) (Mano and Takeda, 1997). In this report, research emphasis was on early vegetative growth of barley, up to the development of the fourth leaf. Tolerance to salinity at the germination stage was not tested here, because the nutrient solution (with or without salts) was used as the unique source of water and nutrients only after emergence of all seedlings. After 2 weeks of salinity exposure, there were visible symptoms of salt damage at the leaf tips (data not shown).

Salinity tolerance of genetic lines can be assessed differently by plant breeders and physiologists. For the former, absolute values of yield/physiological parameters under saline conditions are the most critical to assess agronomic potential (as in Table 3). Under saline conditions at the seedling stage, barley line Patty showed the best overall performance with measured parameters (AGDM, A, g_s, E, F'_v/F'_m, q_P) for which we found significantly different groupings of lines (Table 3). Interestingly, under control conditions, Patty was also the line with the highest values for A, g_s, E, F'_v/F'_m (Figs. 2 and 3). This finding of strong performances in both control and saline conditions is in agreement with a seemingly ambiguous statement by Royo and Aragues (1999), in which they concluded that, for their field conditions, ''the grain yield without salinity was the best statistic for predicting the most productive barley genotypes in salt-affected soils''. For plant physiologists, relative values of growth/physiological parameters, as assessed here by SSIs (Table 4), may be more valuable to understand mechanisms of salinity tolerance. Using this approach, barley lines Sloop, Giza 125 and AZ-8501 seemed to be the most tolerant to salinity as indicated by their respective SSI values regarding parameters for which we found significantly different groupings of lines (AGDM, A, F'_v/F'_m, Δ) (Table 4).

Monitoring gas exchange in plants is a common approach, with stomatal conductance (g_s) reported as one of the most sensitive indicators of stress under salinity for wheat and sorghum (James et al., 2002; Netondo et al., 2004) or progressive drought for grapevine and other C_3 species (Medrano et al., 2002). Reduced C_i for leaves produced under saline conditions has been attributed to stomatal factors predominating over non-stomatal factors in the

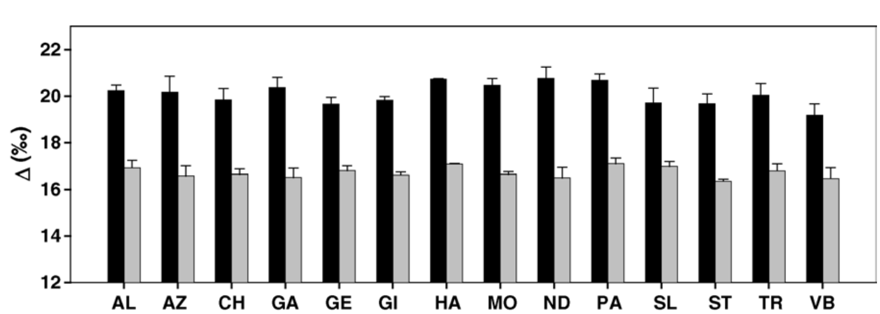

Fig. 5. Carbon isotope discrimination (in ‰) for 14 barley lines in control (black bars) (EC: 3.0 dS m^{-1}) and saline (gray bars) (EC: 20 dS m^{-1}) conditions. Means were calculated from two experiments with five individual plants per experiment. Vertical bars represent two standard errors based on variability among 10 plants. Barley line abbreviations are the same as in Fig. 1.

limitation of CO_2 assimilation activity. Lower C_i should be accompanied by lower stomatal conductance (g_s). However, in some studies (Seeman and Critchley, 1985; Yeo et al., 1985; Rivelli et al., 2002), g_s was lower after exposure to salinity, but C_i did not decrease significantly, indicating that non-stomatal factors were also occurring. Dunn and Neales (1993) reported that C_i was unaffected by salinity in the barley genotype 'Princess' in spite of a reduction of leaf conductance. They concluded that g_s was of secondary importance as mesophyll factors predominated. In our study, a decrease in C_i occurred parallel to decreases in g_s in response to salinity (Fig. 3). Under salinity, g_s was the parameter for which we assessed the largest differences between barley lines (Table 3). Under saline versus control conditions, the decline of g_s was the most pronounced, with g_s SSI ranging from 27 to 46% (Table 4). This impairment of g_s was consistent with that found (i.e., 35%) in field experiments conducted on 34 other barley lines under soil conditions with EC = 22 dS m^{-1} (Isla et al., 1998).

Upon exposure to salinity, the decline in F'_v/F'_m values was minimal compared to that of g_s, as indicated by their respective SSI values (Table 4). Nevertheless, we found a positive relationship between the sensitivity of barley lines to salinity (i.e., SSI values) for g_s and F'_v/F'_m parameters (Fig. 6). In the present study, it is difficult to differentiate between a down-regulation of F'_v/F'_m by stomatal closure, or a co-regulation of these parameters by an undetermined factor. There is a renewed interest in the study of independence or link between stomatal conductance and parameters of photochemical and biochemical efficiencies (Medrano et al., 2002; Flexas et al., 2004). Several studies with various species have cast doubt on the effectiveness of fluorescence parameters for salinity tolerance screening (Lutts et al., 1996; Jimenez et al., 1997; Belkhodja et al., 1999; Ouerghi et al., 2000; Morant-Manceau et al., 2004).

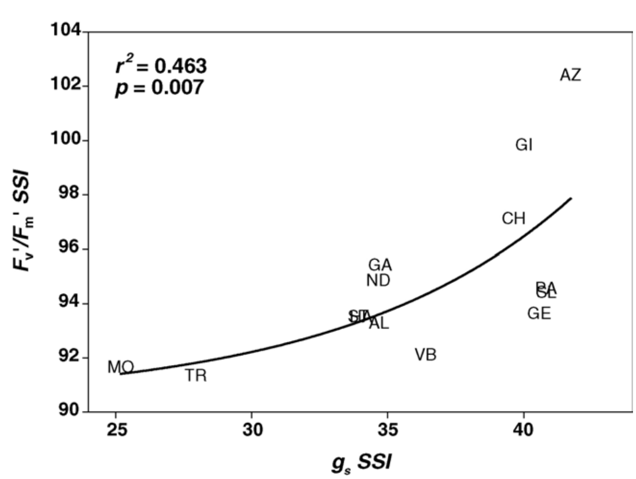

Fig. 6. Relationship between stomatal conductance (g_s) and fraction of absorbed photons used in photochemistry (F'_v/F'_m) of fourth leaf for 14 barley lines. Data are the average of five experiments. Values of salinity susceptibility index (SSI) for g_s and F'_v/F'_m (both in %) are used for axis. Barley line abbreviations are the same as in Fig. 1.

Our results indicated that measurements of F'_v/F'_m were valuable, at least under our experimental conditions and for this developmental stage of barley. There was no decrease in F'_v/F'_m for cultivars previously bred for saline conditions (AZ-8501 and Giza 125) (Table 3), whereas less tolerant lines manifested impairment of PSII, possibly due to ion toxicity (James et al., 2002). Our findings on the relevance of chlorophyll fluorescence for screening tolerance to salinity are in agreement with those made for wheat species, *Triticum aestivum* (KrishnaRaj et al., 1993) and *T. durum* (James et al., 2002). In both wheat studies, differences for fluorescence parameters were found for salt-sensitive lines while salt-tolerant lines remained relatively unaffected. For photochemical quenching (q_P), there was no statistically significant change of values with saline exposure, an indication that the proportion of reaction centers remaining open were similar under control and saline conditions (James et al., 2002).

Carbon isotope discrimination (Δ) was consistently lower under saline conditions (Fig. 5). This observation confirmed previous findings of lower Δ values following exposure to saline growing conditions in tissues of cotton (Brugnoli and Lauteri, 1991), common bean (Seeman and Critchley, 1985; Brugnoli and Lauteri, 1991), cheatgrass (Rasmuson and Anderson, 2002), wheat (Ouerghi et al., 2000) and barley (Isla et al., 1998). Nevertheless, Δ appeared to be of limited value to discriminate between barley lines, especially under salinity.

The magnitude of stomatal/non-stomatal limitations of photosynthesis has been shown to be dependent on the severity of drought stress in wheat (Kicheva et al., 1994). Similarly, Rivelli et al. (2002) have suggested that stomatal factors limiting CO_2 assimilation were observed for intermediate salinity whereas non-stomatal ones occurred at higher salinity. We think that the simplistic approach that leads to a mutual exclusion of either stomatal or non-stomatal factors may be misleading. We found a relationship between the sensitivity of barley lines to salinity for stomatal conductance and a parameter of photochemical efficiency (F'_v/F'_m). Furthermore, two genotypes bred for saline conditions (AZ-8501 and Giza 125) coped well with salt stress, as indicated by their relatively high SSI for A, with two simultaneous attributes, a relatively low impairment of g_s and no impairment of F'_v/F'_m (Table 4).

5. Conclusion

In summary, of all measured parameters and among the 14 barley lines tested here, g_s provided the best information to assess genetic differences for absolute performance under salinity. Lines with the highest g_s values in control conditions also showed some of the highest absolute values for A and F'_v/F'_m under saline conditions. We are pursuing further studies of the relationships between g_s and other gas exchange/fluorescence parameters in barley under both control and saline conditions. For other barley lines, especially those previously bred in presence of soil salinity, some protection of the photosynthetic apparatus was occurring and may be an important factor to their relative tolerance to salinity.

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